Comparative study on enzyme induction and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,4,7,8-pentachlorodibenzofuran and 2,3,4,7,8-pentabromodibenzofuran in marmoset monkeys (*Callithrix jacchus*).

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ABSTRACT

The enzyme inducing potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (**TCDD**), 2,3,4,7,8-pentachlorodibenzofuran (**P5CDF**) and 2,3,4,7,8-pentabromodibenzofuran (**P5BDF**) was investigated in the non-human primate *Callithrix jacchus*. Single doses between 50 and 2400 ng substance per kg body wt. were administered subcutaneously. The enzyme induction was investigated six days after treatment *in vivo* using ^{14}C-labelled caffeine (N3-position) as substrate. One day later the animals were sacrificed and tissue concentrations were determined by GC/MS in liver and adipose tissue. Enzyme induction in hepatic microsomes was studied with ethoxyresorufin as substrate.

TCDD shows a liver/fat ratio of about 1, whereas the chlorinated as well as the brominated pentafuran is deposited mainly in the liver. Enzyme activities were clearly induced after treatment with all three substances. On the basis of the hepatic concentrations a correlation of enzyme activity with tissue concentration was attempted. TCDD was the most powerfull inductor followed by P5CDF and P5BDF. A good correlation was observed between ethoxyresorufin O-deethylase (**EROD**) activity in hepatic microsomes and caffeine N-demethylation *in vivo*. The breath test technique seems to be a useful tool to investigate the enzyme inducing potency of polyhalogenated dibenzo-p-dioxins and dibenzofurans (**PHDD/F**) in the marmoset monkey.

KEYWORDS

Polychlorinated dibenzo-p-dioxins and dibenzofurans; polybrominated dibenzofurans; tissue concentration; enzyme induction

INTRODUCTION

The toxicokinetics of TCDD showed great differences between rodents, non-human primates (marmosets monkeys) and humans[1]. Previous investigations revealed a different response to TCDD determined by measurement of the EROD induction in hepatic microsomes in rats and marmosets[2]. Furthermore, investigations with defined PHDD/F mixtures indicated a different tissue distribution of TCDD and P5CDF in marmosets[3]. P5CDF induced TCDD-like toxicity in Rhesus monkeys[4].
In order to investigate these substances more closely, a study was performed in marmosets using P5CDF and P5BDF and - for comparison - TCDD. The enzyme inducing potency of the three substance was compared *in vitro* and *in vivo* and these values were compared with the hepatic tissue concentration. The comparison of the *in vivo* assay with the enzyme measurement in hepatic microsomes was important, since it is possible to investigate one animal several times using the *in vivo* technique[5].

MATERIAL and METHODS

Chemicals

TCDD (Lot No. 851:142-26) was supplied by Dow Chemical Co., Midland, Michigan, USA. P5CDF and P5BDF were purchased from Promochem, Wesel, FRG). ^{14}C-labelled caffeine was obtained from Amersham Buchler, Braunschweig, FRG, and labelled at the N3-position. All other chemicals used were of the highest purity commercially available (Sigma, Deisenhofen, FRG; Boehringer Mannheim, FRG).

Animal maintenance and treatment

Adult marmosets weighing 330 - 420 g were kept in a colony at a constant day/night cycle. The PHDD/Fs were dissolved in toluene and two volumes of dimethylsulfoxide were added. This solution was injected into the back of the marmosets at a volume of 0.1 ml/kg body wt. The animals were treated either with 50, 150 or 500 ng TCDD/kg body wt. (three animals per dose group), or 240, 800 or 2400 ng P5CDF/kg body wt. (two animals per dose group) or 420 ng P5BDF/kg body wt (three animals). The control animals received the vehicle only.

Experimental procedures

The measurement of the demethylation of caffeine was performed *in vivo* using the breath-test technique, details of the method have been described previously[5]. The investigations were carried out six days after treatment.
Seven days after treatment the marmosets were sacrificed, organ samples were analyzed for the content of PHDD/F by GC/MS[6]. Hepatic microsomes were prepared and the EROD activity was determined[7].

RESULTS and DISCUSSION

The results are compiled in **table 1**. Tissue concentrations in hepatic as well as adipose tissue increase dose-dependently for TCDD and P5CDF. The data for TCDD show no evidence of a dose-dependent change in the liver/fat ratio as described in rats[8]. The high deposition rate of P5CDF in the liver confirm previous investigations

with defined PHDD/F mixtures[3]. The tissue distribution of the brominated congener is similar to that of P5CDF.
The biological activity of TCDD determined by EROD induction levels off at EROD activities of about 1200 pmoles resorufin x mg protein^{-1} x min^{-1} and at hepatic concentrations of > 1 ng TCDD/g liver. Interestingly, this effect is less pronounced for caffeine N-demethylation.
After treatment with P5CDF, EROD activity increases dose-dependently and activities up to 1200 pmoles resorufin x mg protein^{-1} x min^{-1} are achieved. Additional investigations using higher doses would be interesting in order to clarify wether there would be a levelling off phenomenon as observed after TCDD treatment.
EROD induction was less pronounced after P5BDF treatment. When the enzyme activities are compared with the hepatic concentrations, TCDD has the highest potency to induce EROD as well as caffein N-demethylation, the enzyme inducing potency of P5CDF appears to be one order of magnitude lower and that of P5BDF seems to be even less potent.
The toxicokinetics of these compounds must be considered for a evaluation of the potential risk. In previous investigations with marmosets, the elimination half-life in hepatic tissue was 8 weeks for TCDD and 9 weeks for P5CDF[3]. First results on the toxicokinetics of P5BDF are available only for rats. For P5BDF a half-life in hepatic tissue of 99 days was calculated compared to 60 days for P5CDF[9].

Table 1: Concentrations of TCDD, P5CDF and P5BDF in liver and adipose tissue of marmoset monkeys compared with monooxygenase activities.

Congener	Dose [ng/kg]	N	Hepatic tissue [ng/g]	Adipose tissue [ng/g]	Liver/fat ratio	EROD activity*	Caffeine N3-demethylation*
Control	-	3	-	-	-	70 ± 51	0.022; 0.032
TCDD	50	3	0.39 ± 0.33	0.85; 0.32	0.8	660 ± 290	0.078 ± 0.019
	150	3	1.69 ± 0.32	1.71 ± 0.12	1.0	1320 ± 180	0.141 ± 0.011
	500	3	4.49 ± 0.85	5.62 ± 1.10	0.8	1290 ± 230	0.105; 0.150
P5CDF	240	2	5.50; 5.83	0.72; 1.78	5.6	650; 930	n.d.
	800	2	18.8; 21.6	0.88; 1.25	19.3	800; 1050	0.132; 0.155
	2400	2	36.7; 55.7	2.03; 4.52	15.2	1090; 1220	0.134; 0.145
P5BDF	420	3	6.33 ± 0.60	0.56 ± 0.16	12.2	280 ± 27	0.062 ± 0.006

Data are presented as mean ± SD or as single data if less than three values are obtained.
* EROD activity is expressed in pmoles resorufin x mg protein^{-1} x min^{-1}
* Caffein N3-demethylation is expressed in % dose as $^{14}CO_2$/min
n.d. = not determined

The comparison of EROD activity and caffeine N3-demethylation has shown a good correlation and for investigations of inductive effects of dioxin-like substance the *in vivo* technique seems to be useful, especially in studies with non-human primates.

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